UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/786,009	04/17/2001	Ming-Qun Xu	NEB-150PUS	6390

28986 7590 07/03/2002

NEW ENGLAND BIOLABS, INC.
32 TOZER ROAD
BEVERLY, MA 01915

EXAMINER
MOORE, WILLIAM W

ART UNIT	PAPER NUMBER
1652	10

DATE MAILED: 07/03/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	09/786,009	XU ET AL.
	Examiner	Art Unit
	William W. Moore	1652

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *24 January 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *7 and 12-27* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7 and 12-27* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other.

DETAILED ACTION

Priority

The subject matter claimed herein enjoys a priority date of September 30, 1998, being fully disclosed in Applicant's provisional application serial No. 60/102,423, filed on that date, and is similarly disclosed in Applicant's PCT application PCT/US99/22776 of which the instant application is a national stage filing under 35 U.S.C. §371(f).

Information Disclosure Statement

Applicant's Information Disclosure Statement, Paper No. 4 filed April 20, 2001, and citing and supplying all publications cited in the International Preliminary Examination Report, is hereby acknowledged.

Preliminary Amendment and Drawing Figures

Applicant's Preliminary Amendment A, Paper No. 9 filed June 27, 2002, has been entered and claims 1-6 and 8-11 were canceled at Applicant's request. The originally filed claim 7 was amended in Paper No. 9 and the new claims 12-27 were added, thus claims 7 and 12-27 are pending herein. Drawing Figures 1-7 comprised by drawing sheets 1-11 were not reviewed by a Draftsperson, as such review has been discontinued by the USPTO, but the Figures are acceptable for the purpose of examination. Formal Drawings of Figures 2, 3, 6 and 7, when submitted, should be on high-quality photographic paper.

Claim Objections

Claim 18 is objected to because of the following informalities: The word "nucleic" at line 2 of the claim is misspelled.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. §112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 7 is rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 7 is rejected for lack of an adequate written description because the instant specification fails to discuss, exemplify, or describe the preparation of the subject matter of a cyclic protein comprising a target protein ligated to a target peptide with displacement of a thioester tag at the C-terminal of the target protein by formation of a peptide bond with the specified N-terminal portion of a target peptide. Indeed, there is no discussion at all in the specification of production of a cyclic protein or components required for producing one. It is possible that this claim might be advantageously presented in the copending application serial No. 09/249,543, wherein the term "cyclic . . . proteins" is, at least, present, e.g., at page 4 therein. Neither claim 7 nor the present specification describes how a protein and a peptide might be designed to become a cyclic protein or prepared as such. "While one does not need to have carried out one's invention before filing a patent application, one does need to be able to describe that invention with particularity" to satisfy the description requirement of the first paragraph of 35 U.S.C. §112. *Fiers v. Revel v. Sugano*, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993). The Court of Appeals for the Federal Circuit held that a claimed invention must be described with such "relevant identifying characteristic[s]" that the public could know that the inventor possessed the invention at the time an application for patent was filed, rather than by a mere "result that one might achieve if one had made that invention". *University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Nothing in the present specification demonstrates that Applicant was "able to envision" enough of the structure of a cyclic protein produced by fusion or ligation to provide the public with identifying "characteristics [that] sufficiently distinguish it . . . from other materials". *Fiers*, 25 USPQ2d at 1604 (citing *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 18

USPQ2d 1016, 1021 (Fed. Cir. 1991). The absence of discussion, or other treatment, of the claimed subject matter in the present specification will not permit a skilled artisan in the relevant field of protein preparation to predict the structure, or other properties, of a claimed product produced by an undisclosed process.

Claims 7, 12-20 and 22-27 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for:

i) A method of **recombinantly expressing a fusion polypeptide comprising a first selected protein requiring a C-terminal thioester for ligation with a second selected protein or peptide** having an amino-terminal amino acid capable of forming a transient thioester bond which can rearrange to form a peptide bond, **said fusion polypeptide further comprising an intein, or derivative or mutant thereof, fused to the carboxyl-terminus of said first selected protein, wherein said intein, or mutant or derivative thereof, is incapable of promoting protein splicing at its amino-terminal fusion with said first selected protein,** for nucleic acid sequences encoding said fusion polypeptide, vectors and host cells comprising same, and the encoded and expressed first selected proteins thereof having a C-terminal thioester suitable for ligation with a second selected protein or peptide having an amino-terminal amino acid capable of forming a transient thioester bond which can rearrange to form a peptide bond,

ii) A method of **recombinantly expressing a fusion polypeptide**, which may be protein precursor, **which comprises preparing a plasmid wherein a nucleic acid encodes the amino-terminal region of an intein, or derivative or mutant thereof, fused in a single open reading frame to a nucleic acid sequence that encodes a carboxyl-terminus of a protein selected for inclusion in the fusion polypeptide**, which may be protein precursor, **wherein said intein, or mutant or derivative thereof, is incapable of promoting protein splicing at its amino-terminal fusion with said selected protein,**

iii) A method of **activating a protein recombinantly expressed in inactive form by the *in vitro* ligation of a synthetic peptide which restores its activity comprising expression of a fusion polypeptide comprising an inactive, truncated, form of the protein fused at its carboxyl terminus to the amino terminus of an intein, or derivative or mutant thereof, wherein said intein, or mutant or derivative thereof, is incapable of promoting protein splicing at its amino-terminal fusion with said first selected protein,** and **cleaving the inactive form of the protein from the intein in the presence of a thiol reagent to form a protein in inactive form having a C-terminal thioester**, permitting the ligation of a synthetic peptide capable of restoring its activity, said synthetic peptide having an amino-terminal amino acid capable of forming a transient thioester bond which can rearrange to form a peptide bond, and,

iv) A method of **labeling a recombinantly-expressed protein comprising expressing a selected protein fused at its carboxyl-terminus to an intein, or derivative or mutant thereof, which is incapable of promoting protein splicing at its amino-terminal fusion with said first selected protein,**

Does not reasonably provide enablement for a making a cyclic protein or for the practice of any method of claims 12, 17, 22, or 25 with a component other than, at least, an amino-terminal component of an intein incapable of promoting protein splicing at its amino-terminal, including "obtaining an expressed protein with a C-terminal thioester" utilizing a precursor protein that comprises a native intein. The specification does not

enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claim 7 is rejected for lack of enablement because neither the claim nor the present specification describe how a protein and a peptide might be designed to become a cyclic protein, or prepared as such, or the components required for the process. Claims 12-16 are rejected for lack of enablement because claim 12 embraces, in its clause (a), any and all precursor proteins having a native intein where the specification teaches instead that an intein must be specifically modified, by mutating its amino acid sequence or otherwise modifying its structure, e.g., truncating it or dividing it, or both, so that it cannot excise itself with a concomitant protein splicing reaction. Claims 13-16 are included in this rejection because they depend from claim 12 and do not explain how a precursor protein in which an intein does not ordinarily reside can "have" an intein. Claims 17-21, considered to have been intended to describe an invention having statutory utility, are rejected for lack of enablement because claim 17 embraces a method comprising preparation of a plasmid wherein no components which are polypeptide-encoding nucleic sequences are operably-linked in any continuous open reading frame and because the claim further embraces a method utilizing a "cleaving agent" which need not be a an intein, or derivative or mutant thereof, that will fused, upon recombinant expression, to the carboxyl-terminus of a desired region of a "precursor protein", wherein the intein, or mutant or derivative thereof, is incapable of promoting protein splicing with the amino-proximal region of the "precursor protein". Claims 18-20 are included in this rejection because they depend from claim 17 and fail to establish that a plasmid has any polypeptide-encoding nucleic acid sequence regions that are in a continuous open reading frame and because they fail to establish that a proposed "cleaving agent" is an intein, or derivative or mutant thereof, that will be fused, upon recombinant expression, to the carboxyl-terminus of that portion of a "protein precursor" whereby cleavage with a thiol

reagent might form a C-terminal thioester. Claim 21, by contrast, is not included in this rejection because the specification teaches that all necessary protein-encoding nucleic acid sequence elements may operably be linked in the two disclosed pTXB plasmids.

Claims 22-24 are rejected for lack of enablement because claim 22 embraces a method of restoring activity to an inactive protein wherein a "cleaving agent" need not be an intein, or derivative or mutant thereof, that will be fused, upon recombinant expression, to the carboxyl-terminus of the inactive protein whereby cleavage with a thiol reagent might form a C-terminal thioester, and because the claim permits a fragment of any substance to be ligated to restore the activity of the inactive protein. Claims 23 and 24 are included in this rejection because they fail to establish that an intein, or derivative or mutant thereof, will be fused, upon recombinant expression, to the carboxyl terminus of the inactive protein whereby contact with a thiol reagent induces both cleavage of the intein from the inactive protein and the formation of a C-terminal thioester, and because neither claim requires that a "fragment" be, at least, a peptide that, when ligated at the C-terminal thioester will form a peptide bond and restore the activity of the inactive protein. Claims 25-27 are rejected for lack of enablement because claim 25 embraces a method of labeling a protein where a "cleaving agent" need not be an intein, or derivative or mutant thereof, that is fused, upon recombinant expression, to the inactive protein's carboxyl-terminus whereby cleavage with a thiol reagent might form a C-terminal thioester. Claims 26 and 27 are included in this rejection because they fail to establish that an intein, or derivative or mutant thereof, will be fused, upon recombinant expression, to the carboxyl terminus of the protein to be labeled whereby contact with a thiol reagent induces cleavage of the intein from the inactive protein and formation of a C-terminal thioester so that a labeled peptide can label the protein when ligated at the C-terminal thioester.

It is agreed that the scope of enablement provided by the specification, if taken together with the prior art made of record herewith, extends to the use of certain native, i.e., unmodified, inteins in a claimed method only when they are not flanked by native exteins, i.e., when the carboxyl-terminus of the intein is fused to no further polypeptide or peptide component and only the intein amino terminus is fused to the carboxyl terminus of a heterologous protein, such as the proteins Applicant selects in the instant specification and that others have similarly selected, in order to form a C-terminal thioester permitting the ligation of a second selected protein or peptide having an amino-terminal amino acid capable of forming a transient thioester bond which can rearrange to form a peptide bond. This rejection is stated under the first paragraph of the statute because the specification itself teaches away from the use of native inteins in their native context and because neither the prior art made of record herewith nor Applicant's specification suggests that a method of expressing a protein with a C-terminal thioester can be practiced unless an intein that is incapable of conducting excision and protein splicing is fused at its amino terminus to the carboxyl terminus of a selected protein. Neither the specification nor the prior art made of record herewith discloses or suggests a "thiol inducible cleavage agent" other than an intein or a modified intein component.

It is well settled that 35 U.S.C. §112, first paragraph, requires that a disclosure be sufficiently enabling to allow one of skill in the art to practice the invention as claimed without undue experimentation and that unpredictability in an attempt to practice a claimed invention is a significant factor supporting a rejection under 35 U.S.C. §112, first paragraph, for non-enablement. See, *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (recognizing and applying the "*Forman*" factors). Cf., *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (citing eight factors relevant to analysis of enablement). The standard set by the CCPA, the precursor of the Court of Appeals for

the Federal Circuit, requires that a reasonable correlation exist between the scope asserted in the claimed subject matter and the scope of guidance the specification provides. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 25 (CCPA 1970) (scope of enablement varies inversely with the degree of unpredictability of factors involved in physiological activity of small peptide hormone). Applying the "*Forman*" factors discussed in *Wands*, ***supra***, to Applicant's disclosure, it is apparent that:

a) the specification lacks adequate, specific, guidance for conducting a claimed method with a native intein flanked at its carboxyl terminus by a native extein or with a "thiol inducible cleavage agent" other than an intein or a modified intein component,

b) the specification lacks working examples wherein a claimed method is practiced with a native intein flanked at its carboxyl terminus by a native extein or with a "thiol inducible cleavage agent" other than an intein or a modified intein component,

c) in view of the prior art publications of record herein, the state of the art and level of skill in the art does not support practice of such methods.

Thus the scope of the claimed subject matter embraced by the phrases, "obtaining the expressed precursor protein, **the precursor having an intein**", "a fragment" where at least a peptide is required, or "thiol inducible cleavage agent", is considered to be unsupported by the present specification, even if taken in combination with teachings in the prior art.

The following is a quotation of the second paragraph of 35 U.S.C. §112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 7 and 12-27 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 7 is indefinite because, in the absence of any definition to the contrary in the specification, a "cyclic protein" must be construed to be a single, integral, polypeptide having no free amino terminus and no free carboxyl terminus and because the claim fails to explain how two separate components - the protein expressed with a C-terminal thioester and the target protein - will become a single, integral, "cyclic protein". Claim 12 is indefinite in reciting "obtaining", or "obtain", in its preamble and in both of its remaining

clauses, (a) and (b), and because there is no antecedent basis in the preamble or elsewhere for the phrase "**the** expressed precursor protein" in clause (a) of the claim. The term "obtaining" is unambiguous where the preamble of a method claim recites a purpose, "obtaining" some product, and the remainder of the claim provides a set of operations which, performed in a prescribed order, logically produce that product. But the method of claim 12 begins with a product of clause (a) that is "obtained" through no described process and is then reacted with a reagent in clause (b) "so as to . . . obtain" the product desired in the preamble where clause (b) itself does not explain the process and neither the preamble nor clause (a) of the claim describe a structure that permits a reagent of clause (b) "to obtain" the product. Recitation of "obtaining" connotes, poorly, the action of the artisan practicing the method and claim is indefinite because components and processes of clauses (a) and (b) do not, *sua sponte*, "obtain" or conduct themselves. The specification discloses that an artisan actually practices a **method of preparing** an expressed protein with a C-terminal thioester from a recombinantly expressed precursor protein comprising the desired protein fused to an intein that is cleaved upon contact with a thiol reagent. The recombinantly expressed precursor protein is actually the starting material for a disclosed method and claim 12 is further indefinite in failing to explain the nature of "the expressed precursor protein" of clause (a), or where it was "obtained", as well as in failing to explain how it comes to "hav[e] an intein". It is noted that no inteins are "removed" by thiol reagents in methods disclosed in the specification but are instead **cleaved** from contiguous, flanking, polypeptides when **contacted** with such reagents and that this contact is necessary where the disclosed inteins are all unable to conduct protein splicing and have limited, or no, ability to undergo spontaneous cleavage. Claim 12 is additionally indefinite in lacking a terminal recovery or isolation step, leaving the desired product yet to be "obtained" at

the close of the claim's recitation. Claims 13-16 are subject to this rejection in depending from claim 12 but failing to independently resolve the ambiguity of that claim.

Claim 17 is indefinite in failing to describe how a protein precursor can be expressed from a plasmid by virtue of the plasmid's possession of a multiple cloning site and in failing to state a structural relationship between any the apparently unconnected insertions of the various coding regions that might yield a "protein precursor", where the directional term "upstream" cannot require that any of the various coding regions form an open reading frame. Like claim 12, claim 17 is further indefinite where a term, "the cleavage agent sequence", appears in a following clause without antecedent basis elsewhere in the claim. Claim 17 is additionally indefinite in lacking a terminal step wherein a protein is expressed: plasmids alone cannot conduct the expression of proteins. It is also noted that all multiple cloning sites are, of necessity, bound by "two restriction endonuclease sites" because they cannot be bound by one or three, thus the phrase can add no distinguishing element to the description. Claims 18-21 are subject to this rejection because they depend from claim 17 but fail to independently resolve the ambiguity of the claim from which they depend, claim 21 because it cannot provide an expression step.

Claim 22 is indefinite where its preamble recites, "synthetic fragment", because the following steps fail to ligate a fragment to an inactive expressed protein. Applicant may have intended that the preamble recite "synthetic peptide", an element of clauses (b) and (c) of the claim. Claim 22 is further indefinite in reciting, "linked to a thiol inducible cleavage agent", because it lacks an intermediate step wherein such an agent is linked to an inactive expressed protein. It is noted that the specification discloses that it is the thiol reagent that is the catalyst, thus the agent, of cleavage, therefore the claim cannot describe an invention intended by Applicant. Claims 23 and 24 are subject to this rejection because they depend from claim 22 but fail to independently resolve the ambiguity of the

claim from which they depend. Claim 25 is indefinite because it, too, recites, "linked to a thiol inducible cleavage agent", but lacks an intermediate step wherein such an agent is linked to an inactive expressed protein and is contrary to the disclosure of the specification that it is the thiol reagent that is the catalyst, thus the agent, of cleavage. Claims 26 and 27 are subject to this rejection where they depend from claim 25 yet fail to independently resolve the ambiguity of the claim from which they depend.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 12-14, 25 and 27 are rejected under the judicially created doctrine of double patenting over claim 96 of U.S. Patent No. 5,834,247 since the claims, if allowed, would improperly extend the "right to exclude" already granted in the patent. The subject matter claimed in the instant application is fully disclosed in the patent and is covered by the patent since the patent and the application are claiming common subject matter, as follows: A method of making a recombinantly-expressed protein with a C-terminal thioester produced by thiol-agent induced cleavage of a modified Sce VMA intein fused to the desired protein in a precursor, fusion, protein, as well as a method for subsequently labeling the recombinantly-expressed protein with a C-terminal thioester with synthetic peptide comprising a marker which is an antigenic determinant and an amino terminal cysteine.

Furthermore, there is no apparent reason why applicant was prevented from presenting claims corresponding to those of the instant application during prosecution of the application which matured into a patent. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

Claims 7, 12-14, 17-19, 22, 23 and 25-27 are provisionally rejected under the judicially created doctrine of double patenting over claims 1, 7, 15, 16, 32 and 33 of copending Application No. 09/249,543. This is a provisional double patenting rejection since the conflicting claims have not yet been patented. The subject matter claimed in the instant application is fully disclosed in the referenced copending application and would be covered by any patent granted on that copending application since the referenced copending application and the instant application are claiming common subject matter, as follows: A method of making a cyclic protein, which is claim 16 of the copending application, and a method of making a recombinantly-expressed protein with a C-terminal thioester produced by thiol-agent induced cleavage of a modified intein and ligating it to another polypeptide or peptide which may be a second target protein of claim 1 of the copending application which embraces claims 12-14, 17-19, 22, 23 and 25-27 herein in not excluding a synthetic peptide as a ligation partner.

Furthermore, there is no apparent reason why applicant would be prevented from presenting claims corresponding to those of the instant application in the other copending application. See *In re Schneller*, 397 F.2d 350, 158 USPQ 210 (CCPA 1968). See also MPEP § 804.

Claim Rejections - 35 USC §§102 and 103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. §102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in-
>
> (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effect under this subsection of a national application published under section 122(b) only if the international application designating the United States was published under Article 21(2)(a) of such treaty in the English language; or
>
> (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that a patent shall not be deemed filed in the United States for the purposes of this subsection based on the filing of an international application filed under the treaty defined in section 351(a).

Claims 7, 12-14, 16, 25 and 27 are rejected under 35 U.S.C. §102(e) as being anticipated by Comb et al., **U.S. Patent No. 5,834,247**, made of record with Applicant's Information Disclosure Statement.

Comb et al., a patent to another filed eighteen months before the priority date for the instant application, disclose, see Example 19 at cols. 75-77 and claim 96, the expression of precursor polypeptides using the pMYB129 plasmid, see Example 15 at cols. 53-56, from which they obtained expressed proteins having a C-terminal thioester upon thiol reagent-induced cleavage of a modified *Sce* VMA intein fused at its amino terminus to the carboxyl terminus of maltose binding protein and further disclose the preparation of a labeled expressed protein by ligating labeled synthetic peptides having amino terminal cysteines to the C-terminal thioester of the expressed maltose binding protein wherein the labels are peptide fragments which are antigenic determinants, see Figure 29, anticipating claims 12-14, 16, 17, 25 and 27 herein. Comb et al. also disclose, to a greater extent than the present specification, preparation of a cyclic protein, see Example 24 at cols. 87-89 and Figure 37, by generating both a C-terminal thioester tagged protein and a target peptide having a specified N-terminal and ligating the target peptide to the target protein, anticipation claim 7 herein.

Claims 12-14, 17-19, 25 and 26 are rejected under 35 U.S.C. §102(b) as being anticipated by Chong et al., 1997, **Gene**, Vol. 192, pages 271-281, made of record herewith.

Chong et al., June 1997, disclose, see Figures 1, 4 and 5B, and section 2.6 at page 276, the expression of precursor polypeptides using the pCYB plasmid having a multiple cloning site and further providing a nucleic acid sequence encoding a chitin binding protein fused to the cleavage-resistant carboxyl terminus of the modified *Sce* VMA intein, from which they obtained expressed proteins having a C-terminal thioester upon thiol reagent-induced cleavage of a modified *Sce* VMA intein fused at its amino terminus to the carboxyl terminus of a target protein and also disclose preparation of a labeled expressed proteins by ligating a radiolabeled peptide constituting an amino terminal cysteine to the C-terminal thioester of expressed proteins, anticipating claims 12-14, 17, 18, 25 and 26 herein.

Claims 12-14, 17-19, 22, 25 and 26 are rejected under 35 U.S.C. §102(a) as being anticipated by Severinov et al., 1998, **The Journal of Biological Chemistry**, Vol. 273, pages 16205-16209, made of record herewith.

Severinov et al., June 1998, disclose, see procedures at pages 16205-06 and Figure 1, the preparation of a labeled expressed protein using a pCYB2 plasmid having a multiple cloning site comprising a nucleic acid sequence that encoded a linker dipeptide between a carboxyl terminal amino acid of a desired polypeptide and the amino terminus of a modified *Sce* VMA intein, and further providing a nucleic acid sequence encoding a chitin binding protein fused to the cleavage-resistant carboxyl terminus of the modified *Sce* VMA intein, from which they obtained expressed proteins having a C-terminal thioester upon thiol reagent-induced cleavage of the intein – using a variety of the reagents recited in claim 14 herein – to which they ligated a flourescein-labeled peptide comprising a amino terminal cysteine, anticipating claims 12-14, 17-19, 25 and 26 herein. Severinov et al. also disclose, pages 16207-09 and Figure 2, preparation of an inactive expressed protein, the first 566 amino acids of the *E. coli* RNA polymerase σ^{70} subunit, using the pCYB2 plasmid having a multiple cloning site which they obtained expressed inactive proteins having a C-terminal thioester upon thiol reagent-induced cleavage of the modified *Sce* VMA intein to which they ligated a synthetic peptide having an amino-terminal cysteine and the next 34 amino acids of the polymerase σ^{70} subunit, restoring its activity, thus anticipating claims 12-14, 17-19 and 22 herein.

Claims 12-14, 17, 18, 22, 25 and 26 are rejected under 35 U.S.C. §102(a) as being anticipated by Muir et al., 1988, made of record with Applicant's Information Disclosure Statement.

Muir et al., June 1998, disclose, pages 6706-09 and Figures 1-4, preparation of a labeled expressed protein using a pCYB2 plasmid having a multiple cloning site and further providing a nucleic acid sequence encoding a chitin binding protein fused to the cleavage-resistant carboxyl terminus of the modified *Sce* VMA intein, from which they obtained expressed proteins having a C-terminal thioester upon thiol reagent-induced cleavage of the

intein to which they ligated a flourescein-labeled undecapeptide comprising a amino terminal cysteine, anticipating claims 12-14, 17-19, 25 and 26. Muir et al. further disclose that the expressed protein, a C-terminal Src kinase, having a C-terminal thioester upon thiol reagent-induced cleavage of the modified *Sce* VMA was barely active with its native substrates but that ligation of the synthetic undecapeptide having an amino-terminal cysteine and a consensus sequence of conserved activating phosphorylation sites Src kinases greatly augmented its activity, thus anticipating claims 12-14, 17-19 and 22 herein.

The following is a quotation of 35 U.S.C. §103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. §103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. §103(c) and potential §§35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. §103(a).

Claims 12-15, 17, 18 and 23-24 are rejected under 35 U.S.C. §103(a) as obvious over Comb et al. as applied to claims 12-14, 16, 17 above, in view of both Chong et al., 1997, discussed above with reference to claims 12-14, 17 and 18, and Severinov et al., discussed above with reference to claims 12-14, 17-19 and 22.

The disclosure of Comb et al., discussed above, is taken as before and the further teaching of Comb et al., col. 77 at lines 52-54, that "[t]his method can be utilized to synthesize as functional proteins such as enzymes that are toxic to host cells" and this teaching is combined with those of Chong et al., 1997, of preparation of pCYB plasmids for recombinant expression of target proteins including several restriction endonucleases, see Table 1 at page 277 - which are enzymes inherently cytotoxic to any host cell in which they are expressed in the absence of expression of a corresponding, protective, methylase -

fused to a modified *Sce* VMA intein which, in turn is fused via an uncleavable intein carboxyl terminus to the chitin binding protein, permitting ready isolation of the purified restriction endonucleases. The further teaching of Chong et al., 1997, at page 279, left column of text, that "thiol esters that result from intein-mediated cleavage induced by thiol compounds can serve as intermediates in peptide ligation" is also emphasized. Also emphasized are the teachings of Severinov et al. of the division of the amino acid sequence of an enzyme subunit into a larger, inactive, portion for recombinant expression utilizing a pCYB plasmid and subsequent generation of a C-terminal thioester upon cleavage of the modified intein with a thiol reagent together with the solid-phase synthesis of a smaller, peptide portion comprising an amino-terminal cysteine for ligation to the larger, inactive, expressed portion to restore enzymatic activity.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to recombinantly express an inactive, truncated, restriction endonuclease by a method comprising preparing a plasmid according to claims 17 and 18 and obtaining the expressed inactive, truncated, restriction endonuclease with a C-terminal thioester upon cleavage of the *Sce* VNA modified intein with a thiol reagent according to claims 12-15 and to prepare a synthetic peptide comprising the remaining amino acid sequence of the restriction endonuclease with an amino terminal cysteine and to then ligate the synthetic peptide *in vitro* to the expressed inactive, truncated, restriction endonuclease with a C-terminal thioester to restore the activity of the restriction endonuclease. This is because Comb et al. teach that their method should be used to produce toxic proteins and Chong produced restriction endonuclease in low yields with a method similar to, and a plasmid similar to, that of Comb et al. due to the toxicity of these enzymes to the host cells where Chong et al. acknowledge that "thiol esters that result from intein-mediated cleavage induced by thiol compounds can serve as intermediates in peptide ligation" and because

Severinov et al. show how to divide the amino acid sequence of an enzyme into a larger, inactive, portion for recombinant expression utilizing a pCYB plasmid and subsequent generation of a C-terminal thioester upon cleavage of the modified intein with a thiol reagent together with the solid-phase synthesis of a smaller, peptide portion comprising an amino-terminal cysteine for ligation to the larger, inactive, expressed portion to restore enzymatic activity, demonstrating that this method is predictable and efficacious.

Claims 12-14 and 17-19 are rejected under 35 U.S.C. §102(b) as being anticipated by Chong et al., 1997, as applied to claims 12-14 and 17-19 above, in view of Telenti et al., 1997, made of record with Applicant's Information Disclosure Statement.

The teachings of Chong et al., discussed above, are taken as before. Telenti et al. disclose a modified intein that comprises a mutant *Mycobacterium xenopi GyrA* intein that is, see results depicted in Table 1 with the C114R mutant in the "MIEP" expression construct at page 6380, capable of thiol reagent-induced cleavage producing a thioester at the carboxyl-terminus of a polypeptide fused to the intein within a precursor protein which inherently may serve as target protein upon cleavage. It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the modified *Mxe* GyrA intein of Telenti et al. for the modified Sce VMA intein of Chong et al. in practicing the methods of claims 12-14 and 17-19 as they had been practiced by Chong et al. because Telenti et al. demonstrate to such an artisan at that time that their modified *Mxe* GyrA intein is equally capable of producing a thioester at the carboxyl-terminus of a polypeptide fused to the intein within a precursor protein when contacted with a thiol reagent to cleave the intein, thus suitable in the methods of claims 12-14 and 17-19.

Allowable Subject Matter

While subject to rejections above under the first and/or second paragraphs of 35 U.S.C. §112, and requiring amendment to avoid these rejections, claims 20 and 21 are free of the prior art of record herein, and also free of the non-statutory double-patenting

rejections herein, because a sequence search for SEQ IDs NOs:1-4 disclosed no identical or similar linker sequences in the prior art and because no publication discloses the pTXB plasmid before Applicant's priority date for the present application.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 703.308.0583. The examiner can normally be reached between 7:00AM-5:30PM EST on Mondays and Wednesdays, between 7:00AM-1:30PM EST on Tuesdays and Thursdays, and between 8:30AM and 5:00PM EST on Fridays. **The examiner's direct FAX telephone number is 703.746.3169.** If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached at 703.308.3804. Further fax phone numbers for the organization where this application or proceeding is assigned are 703.308.4242 for regular communications and 703.308.0294 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.0196.

William W. Moore
June 27, 2002

NASHAAT T. NASHED PHD
PRIMARY EXAMINER